American Journal of Medical Genetics 69:383–387 (1997)

Telomeric Fusion and Chromosome Instability in Multiple Tissues of a Patient With Mosaic Ullrich-Turner Syndrome

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We describe the cytogenetic evolution of multiple cell lines in the gonadal tissue of a 10-year-old girl with mosaic Ullrich-Turner syndrome (UTS) involving clonal telomeric associations (tas) of the Y chromosome. G-band analysis of all tissues showed at least 2 cell lines; 45, X and 46,X,tas(Y;21)(q12;p13). However, analysis of left gonadal tissue of this patient showed the evolution of 2 additional cell lines, one designated 45,X,tas(Y;21)(q12;p13),−22 and the other 46,X,tas(Y;21)(q12;p13),+tas(Y;14)(q12;p13), −22. Fluorescence in situ hybridization (FISH) analysis of interphase nuclei from uncultured gonadal tissue confirmed the findings of aneuploidy in the left gonadal tissue and extended the findings of aneuploidy to the tissue of the right gonad. The chromosome findings in the gonadal tissue of this patient suggest a preneoplastic karyotype relating to several distinct tumor associations. The clonal evolution of telomeric fusions indicates chromosome instability and suggests the extra copy of the Y chromosome may have resulted from a fusion-related malsegregation. In addition, the extra Y suggests low-level amplification of a putative gonadoblastoma gene, while the loss of chromosome 22 suggests the loss of heterozygosity for genes on chromosome 22. This case demonstrates the utility of the study of gonadal tissue in 45,X/46,XY UTS patients, and provides evidence that clonal telomeric fusions may, in rare cases, be associated with chromosomal malsegregation and with the subsequent evolution of unstable karyotypes. Am. J. Med. Genet. 69:383–387, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: **telomeric fusions; Turner syndrome; gonadoblastoma**

INTRODUCTION

Mosaicism for part or all of the Y chromosome presents a unique problem in patients with Ullrich-Turner syndrome (UTS) and is responsible for one of the few reliable phenotype-karyotype correlations in this disorder, the risk for gonadoblastoma or dysgerminoma [Sybert, 1990]. Females with UTS (gonadal dysgenesis) with bilateral rudimentary streak gonads and a 45,X karyotype do not have an increased risk for gonadal tumors. However, gonadal dysgenesis in a female with UTS, including a 46,XY cell line, is associated with an increased risk of gonadal tumors. Cytogenetic findings in patients with gonadal dysgenesis include 45,X, structural rearrangements of the X or Y, 46,XX (normal female), or 46,XY (normal male) [Verp and Simpson, 1987]. The finding of clonal telomeric associations (tas) and a mosaic 45,X/46,X, tas(Y;21)(q12;p13) karyotype in the blood chromosome studies of this patient prompted the cytogenetic investigation of other tissues. During prophylactic gonadectomy, tissue samples from skin, peritoneum, fascia, and left and right gonads were obtained for cytogenetic analysis. Tissue from the dysgenetic gonads contained 4 distinct cell lines, including the 2 found in the blood and 2 which were found in no other tissue, i.e., 45,X,tas(Y;21)(q12;p13),−22 and 46,X,tas(Y;21)(q12;p13),+tas(Y;14)(q12;p13),−22. The findings of an additional Y chromosome, monosomy for chromosome 22, and clonal telomeric fusions have not been previously reported in the gonadal tissue of a patient with a mosaic Turner karyotype.

CLINICAL REPORT

The patient was initially referred at 10.6 years for short stature. She was in good general health, but had

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Received 5 March 1996; Accepted 5 August 1996

grown inadequately for the past 3 years. Past medical history was significant for seasonal allergies, chronic otitis media, and occasional urinary tract infections. Birth weight was 2.8 kg (~5 centile). The patient lived with her biological father and step-mother. Paternity testing had previously been performed with HLA typing confirming the biological father. On physical examination she appeared healthy with a height of 129.3 cm (<5 centile) and weight 34.7 kg (<50 centile). She had a low posterior hairline, high arched palate, mild cubitus valgus, hyperconvex nails, and Tanner I breast and Tanner II pubic hair development. Additional evaluations documented normal complete blood count (CBC), electrolytes, thyroid screen, urinalysis, bone age, and insulin-like growth factor-1 (IGF-1) values. Gonadotropins were elevated at luteinizing hormone (LH; 8.0) and follicle-stimulating hormone (FSH; 56). L-Dopa-stimulated growth hormone was 15.0. Pelvic and renal ultrasonograms were obtained and showed a small uterus and ovaries. The kidneys and collecting ducts were normal. An echocardiogram disclosed a bicuspid aortic valve with mild stenosis and very mild insufficiency. Left ventricular size and function were normal. There was no evidence of coarctation of the aorta. Because of the Y-bearing cell line and the presence of intra-abdominal gonads, a gonadectomy was recommended. At the time of surgery, tissue samples were obtained from the right gonad, left gonad, peritoneum, fascia, and skin.

PATHOLOGY FINDINGS

The right and left salpingo-oophorectomy specimens measured 2.0 × 1.5 × 1.3 cm and 2.0 × 1.0 × 0.8 cm, respectively. Histologic findings for the two gonads were similar, consistent in each case with ovarian dysgenesis. These findings included a streak of sworled, fibrous ovarian-type stroma containing scattered epithelial inclusion cysts of variable size, with adjacent areas of adipose tissue. The latter contained numerous vascular structures as well as occasional small islands of clustered epithelial cysts with a cuboidal to columnar lining, associated with disorganized bundles of smooth muscle cells. There was no histologic evidence of malignant transformation in the tissue examined.

CYTOGENETIC FINDINGS

Analysis of 30 trypsin-Giemsa banded metaphase cells from phytohemagglutinin (PHA)-stimulated peripheral blood leukocyte cultures [Yunis et al., 1978] from the proposita showed 15 cells (50%) with a 45,X chromosome pattern and 15 cells (50%) with a 46,X,tas(Y;21)(q21;p13) chromosome pattern (Fig. 1). Chromosome nomenclature and findings are described in accordance with ISCN 1995 [Mitelman, 1995]. Chromosome studies of the father's blood showed a normal 46,XY karytoype with no evidence of tas on the Yq.

Tissue samples were transported directly from surgery to the cytogenetics laboratory and treated as previously described [Sawyer et al., 1992]. Abnormal findings for tissue analysis were confirmed in at least 6 different primary cultures. Cultures from skin showed

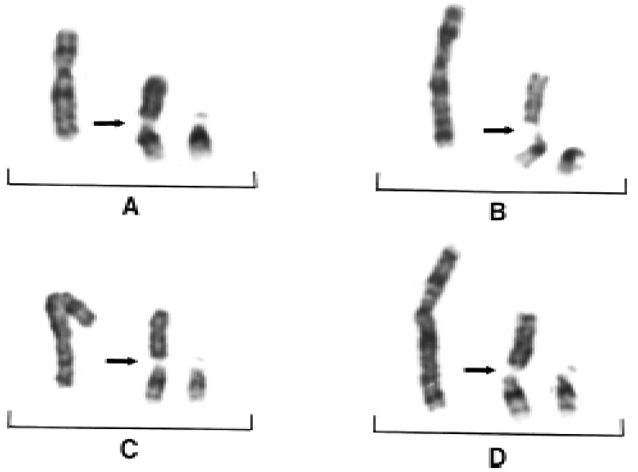

Fig. 1. Partial karyotypes of 4 representative G-band cells (**A-D**) from peripheral blood chromosome studies showing variations in the levels of fusion of Yqter and 21pter (arrows indicate fusion points).

35 cells (70%) with a 45,X and 15 cells (30%) with a 46,X,tas (Y;21)(q12;p13) karyotype. Fascia demonstrated 44 cells (88%) with a 45,X karyotype and 6 cells (12%) with a 46,X,tas (Y;21)(q12;p13) karyotype. Cultures from the peritoneum revealed 42 cells (84%) with a 45,X and 8 cells (16%) with a 46,X, tas (Y;21)(q12;p13) karyotype. Cultures from right gonadal tissue demonstrated 45 cells (90%) with a 45,X and 5 cells (10%) with a 46,X, tas (Y;21)(q12;p13) karyotype. Cultures from left gonadal tissue: 23 cells (46%) with a 45,X, 3 cells (6%) with 46,X, tas (Y;21)(q12;p13), 20 cells (40%) with 45,X,tas(Y;21)(q12;p13),−22 (Fig. 2A, B), and 4 cells (8%) with 46,X,tas(Y;21)(q12;p13), +tas(Y;14)(q12;p13),−22 (Fig. 2C, D).

Fluorescence in situ hybridization (FISH) of the blood and uncultured gonadal tissues were used to confirm and extend the findings of the conventional cytogenetic studies. FISH of the blood with a Y chromosome cocktail probe (Oncor Inc., Gaithersberg, MD) confirmed the presence of alpha satellite (DYZ3) and classical satellite sequences (DYZ1) on the Y in this patient (Fig. 3A). An all-telomere probe (Oncor) to the Y;21 fusion chromosome showed telomeric sequences present at the fusion point of the 2 chromosomes (Yqter and 21pter) and at both of the unfused telomeres (Ypter and 21qter; Fig. 3B). FISH studies with the Y cocktail probe and an alpha satellite probe for chromosome 22 (Oncor, 14/22 alpha satellite probe) were done on interphase nuclei of direct preparations of gonadal tissue to confirm the findings of the in situ cultures. Interphase nuclei from 200 cells (direct preparation) were examined for the presence of a Y signal. Data from 200 interphase cells of a normal control (46,XY) showed 1% with no signals, 98.5% with one signal, and .5% with 2 signals. In the left gonadal tissue, 94 nuclei (47%) showed no Y signals (45,X cell line), 99 (50%) showed one signal (XY cell line), and 7 (4%) showed 2 signals (YY cell line), (Fig. 3C). In the right gonadal tissue, no signals were seen in 93 nuclei (47%), one signal was seen in 99 nuclei (50%), and 2 signals were seen in 8 nuclei (4%). For the 14/22 alpha satellite probe, 100 nuclei from both gonadal tissues were ex-

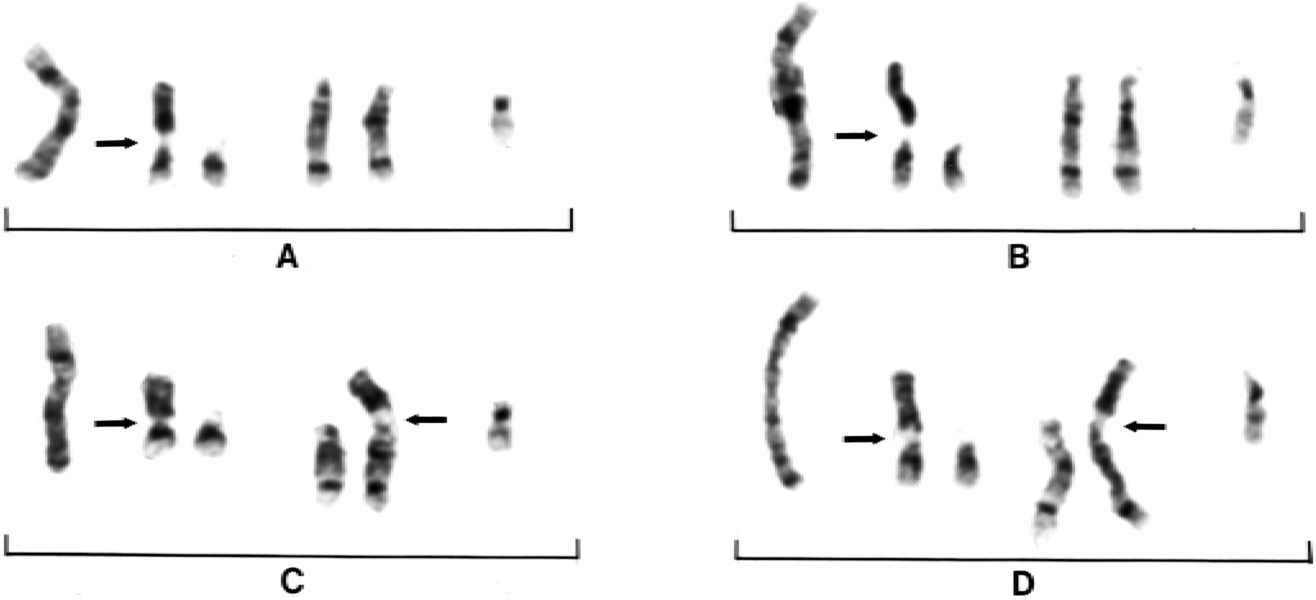

Fig. 2. Partial karyotypes of 4 G-band cells from left gonadal tissue (**A-D**) showing clonal evolution of telomeric fusions and aneuploidy in the evolution of the variant cell lines (arrows indicate fusion points). Two cells (A, B) showing clonality of X,tas (Y;21)(q12;p13) and monosomy 22 cell line. Two cells (C, D) showing the X,tas (Y;21)(q12;p13),tas (Y;14)(q12;p13),−22 cell line (arrows indicate fusion points).

amined. Data from 200 nuclei from a normal control (46,XY) showed 1% with 2 signals, 5% with 3 signals, and 94% with 4 signals. In the left gonadal tissue, 56 nuclei showed 3 signals (56%) (monosomy 22) (Fig. 3D) and 44 nuclei showed 4 signals (44%). From the right gonadal tissue, 3 signals were seen in 50 nuclei (50%) and 4 signals were seen in 50 nuclei (50%). The apparent discrepancy between the G-banded results (cultured cells) and FISH studies (uncultured cells) in the right gonadal tissue may be partially explained by the propensity for "normal" cells to proliferate faster than abnormal cells in culture. These results confirmed the findings of + Y and monosomy 22 in the cultured tissue of the left gonad, and extend the pattern of aneuploidy to the right gonad.

DISCUSSION

There is a high risk of neoplasia in patients with gonadal dysgenesis and 45,X/46,XY mosaicism [Sybert, 1990]. Therefore, the finding of the Yqter involved in a telomeric fusion with 21pter in the blood chromosome studies of this patient implies a risk for subsequent chromosome instability. Telomeric fusion is a phenomenon in tumors in which the telomeres of a single chromosome or 2 distinct chromosomes are associated or fused with minimal or no loss of material from either chromosome end. The significance of telomeric fusions remains obscure, although they clearly represent a form of chromosome instability present in pathologic tissues and are associated with ring chromosome formations and subsequent chromosome loss [Schwartz et al., 1990; Sawyer et al., 1992, 1993a, b, 1994, 1995]. In normal cells, telomeric repeats appear to be necessary for chromosome stability, and therefore, telomere loss or shortening results in fusion events and chromosome instability [Hastie and Allshire, 1989; Hastie et al., 1990]. The mechanism by which tas causes deletions and structural rearrangements occurs as the result of breakage and fusion cycles between centromeres of dicentric chromosomes [McClintock, 1941]. Dicentric chromosomes originating from telomeric fusions tend to pull apart during mitosis and can result in deletions and unbalanced segments due to breakage between the 2 centromeres.

The clonal evolution of variant cell lines in the gonadal tissue of this patient is demonstrated by 4 distinct chromosomal events. The first event was the fusion of the telomere of the Yqter and 21pter (Fig. 1). This aberration was found in all tissues examined, and therefore, was apparently present from early embryogenesis. The second step in the evolution of the subsequent subclones was the loss of chromosome 22 in the Yqtas containing stemline (Fig. 2A, B). The third event, and the most intriguing finding, was the appearance of a subclone showing not only the −22, but also a second Y. We think the clonal telomeric fusions of Yqter may have somehow been a factor in the malsegregation of the Y chromosome. The disruption in the mitotic spindle is believed to be a common mechanism for nondisjunction, but in this case one could speculate that the configuration of tas Yq;21p somehow posed a problem at anaphase resulting in the nondisjunction of the Y. By whatever mechanism it occurred, it is clear that the qter of the second Y chromosome then became fused with 14pter, representing the fourth event observed in this progression (Fig. 2C, D). The clonal fusion of the second Y chromosome with a chromosome 14 suggests the Yq telomere is in some way defective and has a propensity for fusion. This type of clonal karyotypic evolution of abnormal cell lines is characteristic of chromosome instability commonly seen in solid tumor evolution. Unfortunately, very few tumors from mosaic

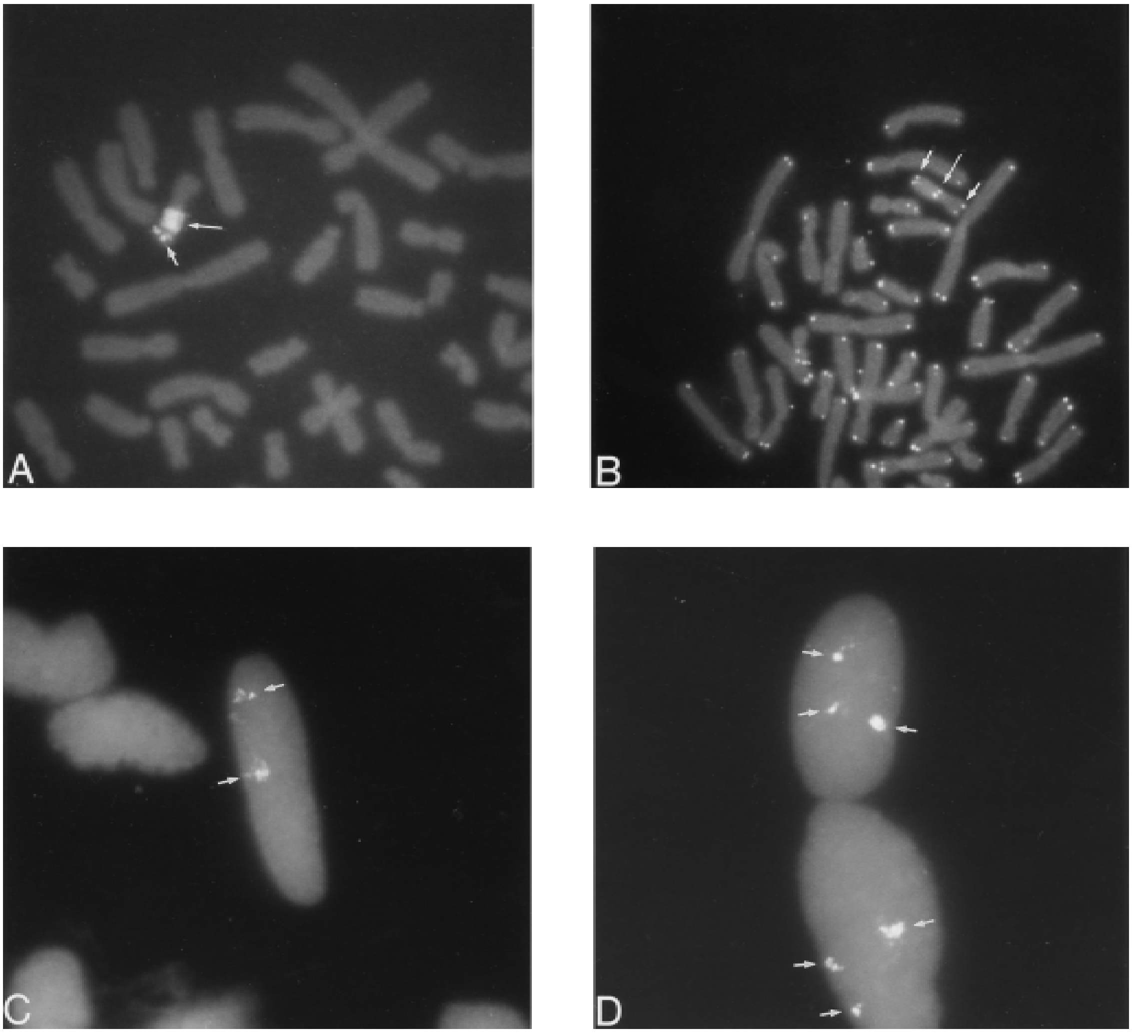

Fig. 3. **A:** Metaphase spread from peripheral blood showing hybridization of Y chromosome cocktail probe. Short arrow indicates alpha satellite sequences (DYZ3) and longer arrow indicates classical satellite sequences (DYZ1) to the Y. Note fusion of 21p to Yqter. **B:** Metaphase spread from blood showing telomeric sequences on the short arm of Yp (short arrow, left), fusion point of Yq and 21p (long arrow, middle), and telomere of 21q (short arrow, right). **C:** Y cocktail probe to interphase nuclei from direct harvest of left gonadal tissue showing 2 copies of the Y (arrows). **D:** Hybridization of a 14/22 probe (arrows) to a direct harvest of left gonadal tissue showing 3 copies in 2 cells (monosomy 22).

UTS patients have been examined for chromosome aberrations, and those that have been studied have shown the same karyotype as the somatic cells [Sandberg, 1990].

The chromosomal imbalance present in the gonadal tissue in this patient suggests the involvement of at least 2 categories of cancer-relevant genes. The extra Y chromosome implies 2 copies of a putative gonadoblastoma gene, while the loss of chromosome 22 implies the loss of heterozygosity for a gene on this chromosome. At the present time, the origin of tumors in these patients centers on the presumption that particular Y-chromosomal genes predispose girls and women with dysgenetic gonads to the development of gonadoblastomas. The identity of the so-called gonadoblastoma gene has not been established; however, Page [1987] has postulated a gonadoblastoma gene (GBY) on the Y chromosome, with current evidence implicating the middle portion of the chromosome at or near the centromeric region of the Y [Page, 1994; Tsuchiya et al., 1995]. The effect of having 2 copies of the GBY gene in the gonadal tissue of this patient is unknown, but could conceivably result in a low-level dosage effect for gene amplification. Loss of the Y chromosome is common in hematologic disorders; however, the gain of a Y chromosome in solid tumors is rare [Mitelman, 1991], and therefore, its presence is highly suggestive of a pathologic condition. If a GYB gene exists on the Y chromosome it would provide further support for the hypothesis that single extra copies of a gene produced by nondisjunction or other duplication mechanisms are important in some cases of tumor development [Nowell, 1989].

The finding of −22 in cells of the gonadal tissue is consistent with a common type of genomic imbalance in solid tumors, the loss of tumor suppression (Fig. 2A-D). Molecular and cytogenetic evidence indicates chromosome 22 harbors tumor suppressor genes for several different tumor types, including meningioma, acoustic neuroma, ependymoma, and low-grade gliomas [Wein-

berg, 1991]. In addition, monosomy of 22 is also a frequent finding among the complex abnormalities seen in ovarian tumors [Page, 1994; Jenkins et al., 1993; Dobson et al., 1993; Heim and Mitelman, 1995], including granulosa cell tumors of the ovary, a well-known "borderline" neoplasm [Leung et al., 1990]. Recent molecular studies suggest that a tumor suppressor gene on chromosome 22 distal to the NF2 gene is involved in ovarian cancer [Cambell et al., 1995; Dodson et al., 1995]. Interestingly, tas have been reported in at least one study of advanced ovarian carcinoma, with 15 of 20 tumors (75%) examined showing telomeric associations [Mrozek and Limon, 1992].

Chromosome instability is recognized as one of the hallmarks of the tumor phenotype. The analysis of pathologically defined tumors has allowed the multistep nature of cancer to be defined and to be correlated, in some respects, with increasing clonal chromosome instability. The defining of clonal chromosome aberrations in tissue with an increased propensity for tumor development, such as the gonadal tissue in this case, may help identify new tumor-related chromosome aberrations, and therefore, could have significant implications in diagnosis and treatment. The cytogenetic findings described here suggest the examination of gonadal tissue with both conventional and FISH analysis of these patients may provide clues to the oncogenic process. The combination of the findings of telomeric fusions, +Y and −22, occurring together in the gonadal tissue, we believe, is evidence of the presence of preneoplastic cells. Tumor cells are known to be more genetically unstable than comparable normal cells and thus may have an increased propensity for the generation of variants, leading to emerging subpopulations and evolving biological and clinical phenotypes [Nowell, 1989]. Since structural changes in the genome or chromosome constitution are not readily susceptible to reversibility, it seems likely that the aneuploidy seen in gonadal tissue would have a high probability for subsequent variants, thus indicating changes consistent with neoplastic transformation.

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